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Cont

66. (New) The recombinant protein preparation of claim 63, wherein the analog comprises less than 3 amino acid substitutions.--

Remarks

Introductory Comments:

Claims 25-27 and 43-45 and 55-57 were examined in the Office Action dated May 18, 1998 and rejected based solely on 35 USC §102(b), as anticipated by Heldin et al., *Nature* (1986) 319:511-514 ("Heldin"). This rejection was traversed in the paper filed by applicants on November 18, 1998. However, the Advisory Action maintained the rejection, stating that the response presented arguments "previously presented and answered." Accordingly, applicants submit herewith further arguments and evidence to support their position.

Overview of the Above Amendments:

Claims 25-27 have been amended to eliminate the terminology "substantially homologous and functionally equivalent" and to substitute instead the recitation that the analog "comprises less than 10 amino acid variations and retains PDGF biological activity as measured using a human foreskin fibroblast mitogen assay." These amendments are made solely to recite the invention with greater particularity and are not meant to be an acquiescence in any rejections.

New claims 58-66 have been added and recite particular embodiments of the invention.

Support for the amendments and new claims may be found throughout the specification at, e.g., page 6, lines 24-31 and page 38, line 16 through page 39, line 28. Thus, no new matter has been added to the application.

Rejection Under 35 USC §102(b):

The rejection of claims 25-27, 43-45 and 55-57 under 35 U.S.C. §102(b), over Heldin et al., *Nature* (1986) 319:511-514 ("Heldin") was maintained in the Advisory Action, apparently for reasons of record. In the final rejection, the Office asserted that Dr. Betsholtz's Declaration was insufficient to overcome the rejection allegedly because certain statements made therein were "not supported with any evidence of record." In particular, the Office Action disputed statements made in paragraph 5 of the Declaration that "methods of purifying proteins from human sources...cannot result in a protein product free of contaminating human proteins." The Action argued that Heldin's preparation is purified to homogeneity because: (1) "Heldin et al. demonstrate a single band on a silver-stained gel, which is indicative in the art of a homogeneous preparation of protein" (Office Action, page 4, lines 1-2); (2) "Heldin et al. specifically state that 'one homogeneous component of M_r 31,000 was obtained in non-reducing conditions'... The "Declaration has identified no human virus which would copurify with a molecular weight of 31,000" (Office Action, page 4, lines 3-5); and (3) "Heldin et al. state that no other amino acid sequence was obtained from the purified PDGF AA preparation" (Office Action, page 4, lines 5-6) .

Applicants do not agree with the above contentions and are submitting the Declaration of Lawrence Scott Cousens, Ph.D., in order to address each of these allegations. In particular, as stated in paragraph 2 of Dr. Cousens' Declaration, none of the above criteria can be used to support the conclusion that Heldin's preparation was free from human proteins other than the ODGF protein.

First of all, as explained in paragraph 3 of Dr. Cousens' Declaration, "no purification technique that uses a human cell as the source of the protein, can render a preparation which is absolutely homogeneous and lacking in other human proteins...because no protein purification technique is capable of providing a completely

pure product." Dr. Cousens supports this statement with an excerpt from an article written by Dr. Arthur Kornberg, a highly acclaimed Nobel Prize recipient:

> No enzyme is purified to the point of absolute homogeneity. Even when other proteins constitute less than 1% of the purified protein and escape detection by our best methods, there are likely to be many millions of foreign molecules in a reaction mixture. (p. 2, of Exhibit B, attached to Dr. Cousens' Declaration, emphasis added.)

It is Dr. Cousens' conclusion then, that "a protein purified from human starting material must, by necessity, include other human contaminants, even if these contaminants are undetectable in a silver-stained gel." See, paragraph 3 of the Declaration. This, in and of itself is sufficient to distinguish the present claims from Heldin.

Additionally, in paragraph 4 of the Declaration, Dr. Cousens explains that a number of proteins do not even stain using silver stains! Further, proteins which may in fact be present in a preparation can easily escape detection. Dr. Cousens states that "some low molecular weight proteins may diffuse out of the gel and not be stained at all, some may stain very weakly, and others may stain disproportionately strongly." It is Dr. Cousens' conclusion that "a single band on a silver-stained SDS polyacrylamide gel, as used in Heldin, does not prove that the product is homogeneous...Had the gel shown in Figure 2 of Heldin been exposed longer, different stains or staining conditions been used, or other methods of protein detection been utilized, human protein contaminants would most certainly have been seen." Thus, the absence of protein bands on a silver-stained gel is not at all indicative that contaminating human proteins were in fact absent.

Moreover, as explained in paragraph 5 of Dr. Cousens' Declaration, viral contaminants in protein products obtained from human sources are often difficult to detect and do not generally show up with silver staining. In fact, transmittive electron microscopy (TEM) appears to be considered the most sensitive measure of viral

contamination by the FDA, and even using this method, the FDA assumes the titer of virus present in a protein preparation to be equivalent to the lowest limit of detection, i.e., 1,000,000/ml. See, Exhibit D attached to Dr. Cousens' Declaration. Therefore, Dr. Cousens concludes that "it is entirely possible that viral contaminants are present in Heldin's preparation, including contaminants that do not 'copurify with a molecular weight of 31,000.'"

Applicants wish to clarify that at no time have they stated that Heldin's preparation in fact includes viral contaminants, as alleged in the Action. Rather, applicants have asserted that Heldin's preparation could well be contaminated with undetectable human viruses due in part to the fact that the cell line from which ODGF was isolated was established from a human patient suffering from cancer. Thus, the cell line may have contained pathogenic viruses from the patient. Additionally, since the cell line was of human origin, it could easily become infected with human pathogenic viruses during propagation.

Finally, as explained in paragraph 6 of Dr. Cousens' Declaration, just because no other amino acid sequence was obtained from the Heldin preparation does not mean that contaminants were absent. It is well known that N-terminal sequencing will not detect contaminants with "blocked" amino termini and many eukaryotic proteins, including some human proteins, are blocked.

Based on all of the foregoing, it is evident that Heldin's preparation cannot be free of human protein contaminants. This statement is supported on the record by two Declarations of prominent scientists in the field, including the Declaration of a coauthor on the Heldin paper! Further, this statement is supported by an article written by renowned Nobel Laureate, Dr. Arthur Kornberg. The Office's position directly contradicts these statements, statements made by scientists clearly skilled in the relevant art. Drs. Betsholtz's, Cousens' and Kornberg's statements are therefore highly probative.

Applicants respectfully request that if the Examiner maintains the assertion that the evidence is inadequate to overcome the instant rejection, she present her qualifications as one of ordinary skill in the art in an affidavit pursuant to 37 CFR §1.107(a), along with facts to support the conclusion. Without such a showing, the rejection cannot stand.

Finally, the Advisory Action states that applicants' arguments were previously presented and answered. However, in the response to the final rejection, applicants pointed out how the preparations of claims 55-57 differed from Heldin. The Office has not explained why this rejection has been maintained. Applicants reiterate that claims 55-57, directed to preparations for topical administration, are not anticipated by Heldin. As explained above, Heldin's preparation was not completely devoid of other human proteins. Additionally, the disclosure of phosphate buffer, relied on by the Office, is with reference to subparts (a) and (b) of Figure 1. Figures 1(a) and 1(b) clearly relate to heterogeneous mixtures of proteins, as evident from the multiple bands seen in the figures. Accordingly, the legend of Figure 1 does not render claims 55-57 anticipated, as argued by the Office.

Applicants reiterate it is only because they cloned the gene encoding PDGF that they were able to prepare a PDGF preparation free of other human protein contaminants. A significant advantage is gained by producing the proteins recombinantly rather than by isolating PDGF from natural sources as preparations can be obtained that are devoid of molecules normally present in cells that naturally produce the protein. Potential viral agents from natural sources are also avoided. Thus, a preparation containing a recombinant protein both distinguishes from, and has significant advantages over, the prior art. Applicants therefore request withdrawal of the rejection over the art.

Conclusion

Applicants respectfully submit that the claims define an invention which is novel and nonobvious over the art. Accordingly, allowance is believed to be in order and an early notification to that effect would be appreciated.

Please direct all further communications in this application to:

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Respectfully submitted,

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